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SEMI-ANNUAL PROGRESS REPORT

To The

Biochemistry Branch-Office of Naval Research

Contract Nonr-859(00)

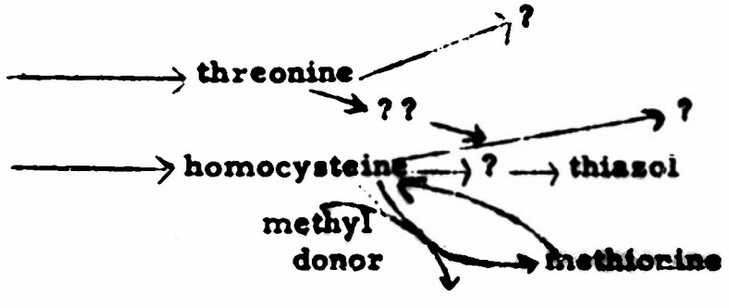
For Biochemical and Genetical Research on Certain Mutants of Neurospora

The University of Texas

Submitted by: Robert P. Wagner, Principal Investigator,
January 13, 1953.

Scientific Progress: Substantial progress has been made in the following three directions: (1) studies on mutants inhibited by threonine and histidine, (2) crosses between biochemical mutants, and (3) a study of intracellular free and bound amino acids of certain biochemical mutants. In addition a study of the effect of trace elements on the expression of nutritive requirements of several mutant strains has been undertaken, and a mutant strain which requires both phenylalanine and tyrosine for complete growth and accumulates considerable quantities of an unknown (possibly aromatic) compound in the medium.

Preliminary work on the threonine inhibited mutant (T 77) had already been published prior to the beginning of this contract (Doudney and Wagner, Proc. Nat. Acad. Sci. 38:196-05, 1952). A synopsis of some of the significant growth characteristics of this mutant as compared to the wild type is given in the accompanying table which includes published data as well as results of experiments carried out since the beginning of the contract. These data together with the observation that T 77 growing in the presence of threonine is low in methionine and thiazol (or thiamin) content have led us to the following tentative picture of this area of metabolism.



It is assumed that the same scheme holds for both the mutant and the wild type,

TABLE

Growth Characteristics of T 77 as Compared to Wild Type (WT)

	Temperature	Minimal	Threonine (10^{-3} M or less)	Homocysteine or Methionine	
				Low Conc. (10^{-4} M)	High Conc. (10^{-3} M to 10^{-2} M)
T 77	25° C	50% WT	inhibited, inhibition completely relieved by methionine, homocysteine, homoserine, sulfanilamide, partially by choline, completely by choline + thiazol	stimulate to 100% WT	inhibited, inhibition relieved by threonine
T 77	35° C	100% WT	inhibited severely, inhibition completely relieved by same compounds effective at 25° C	no effect 100% WT	inhibited, but less than WT; inhibition relieved by choline + threonine
Wild Type	25° C	----	no effect	no effect	not det.
Wild Type	35° C	----	no effect	no effect	complete inhibition at 10^{-2} M, relieved by choline alone

but that the mutant has inadequate control over the reaction between threonine or one of its products and homocysteine or product such that an excessive amount of threonine causes a reduction in the amount of homocysteine available for the two other reactions indicated in the scheme involving homocysteine.

Crosses have been made between T 77 and a number of threonine and methionine requiring strains. Three of these combinations show interesting interactions and are being intensively investigated at the present time. In one of them the threonine inhibition is partially suppressed, in the other two it is enhanced.

Experiments using extracts of T 77 mycelium show that this strain produces a substance which inhibits the growth of wild type, but only in the presence of threonine. These results are of extreme interest to us in our search for the mechanisms underlying the formation of genetic blocks.

A second mutant strain (T 66) inhibited by an amino acid, but which otherwise grows like wild type on minimal medium is being investigated. This strain is severely inhibited by histidine (10% WT growth on 10^{-4}M histidine) and the inhibition is relieved by any one of a variety of amino acids, including those known to be important in transamination. Enzyme studies with extracts from the histidine inhibited strain and wild type show that histidine inhibits transamination in Neurospora when x-ketoglutarate is used as one of the substrates. The inhibition in these in vitro experiments by histidine is, however, equivalent for both mutant and wild type enzyme extracts. On the other hand, other experiments show that histidine is causing an upset in amino acid metabolism or nitrogen utilization which it does not cause in wild type, and the investigation into the basis of the malfunction is being continued.

The immediate purpose of the two investigations described above is to determine the difference between inhibited strains and wild type. The work is predicted on the hypothesis that inhibited strains are possibly intermediate types of nutritional mutants. If the mutation were of a more drastic type than in the case of the threonine inhibited strain, for example, the strain would be inhibited by its own threonine and hence classed as a methionineless mutant caused by the absence or alteration of an enzyme directly involved in methionine synthesis. Hence studies

of inhibition caused by naturally occurring compounds may well lead to a better understanding of the internal control of metabolism.

In addition to the synthesis of double mutant strains between the threonine inhibited strain and various methionine and threonine requiring mutants as described above crosses are also being carried out with the pantothenicless strain and mutants which have a block in aromatic synthesis or acetate metabolism. A number of double mutants have been made, but little biochemical work has been done with them up to the present writing.

As part of the general program of attack on elucidating the nature of genetic blocks in biochemical mutants, we have started a chromatographic analysis of the free and bound cellular amino acids and other ninhydrin positive substances in the mycelia of the mutant strains of most interest to us in our other investigations. Preliminary work has shown some marked differences between these and wild type, but we are currently backcrossing all mutants for five generations with wild type to attain approximate isogenicity before arriving at any definite conclusions.

Change in Direction or Emphasis: There has been no marked change in direction in our thinking up to the present time, but we have shifted our emphasis somewhat from a consideration of the more orthodox biochemical mutants to inhibited mutants. An analysis of these we believe may yield information which will aid us to better analyze and understand those mutants with complete blocks and consequent increased requirements.

Publications: No manuscripts have been sent to press since the beginning of the contract, but two are in the course of preparation-- one on further characterization of the growth requirements of T 77 and a second on the phenomenon of histidine inhibition of transamination.

Personnel: Besides the Project Director, R. P. Wagner, the following are employed in working on the project problem part time:

Charles Doudney, B.A., predoctoral research assistant.
James Ragland, B.A., M.A., predoctoral research assistant.
Robert Fuerst, B.A., M.A., predoctoral research assistant.
A. Gib DeBusk, B.A., M.A., predoctoral research assistant.
Anne P. Doudney, B.A., research assistant.

The first four are graduate students working toward the Ph. D. degree in biochemical genetics under the project director. Mr. Doudney is expected to complete his degree by June of 1953 and will be replaced by another graduate student if the contract is renewed. All four of these students have been on contract funds since the beginning of the contract on June 16, 1952. Mrs. Doudney was employed on contract funds as a technical assistant from [illegible] to August [illegible]. [illegible] September she has been employed in the same status as an assistant to the Project Director on Rockefeller Funds aiding him in his work on the project problem.

The Project Director, R. P. Wagner, received compensation for two months from June 16, 1952, to August 16, 1952. The original contract specifications stated that he was to receive compensation for [illegible] months, but he decided to forego the extra month's compensation in order that an additional graduate student might be employed on the problem.

In addition to funds supplied by the Office of Naval Research, work on this project is aided by funds from the Rockefeller Foundation which have been used particularly for the purchase of permanent type equipment needed in the research, and to pay for the continued services of the technical assistant, Mrs. Anne P. Doudney, as stated above.